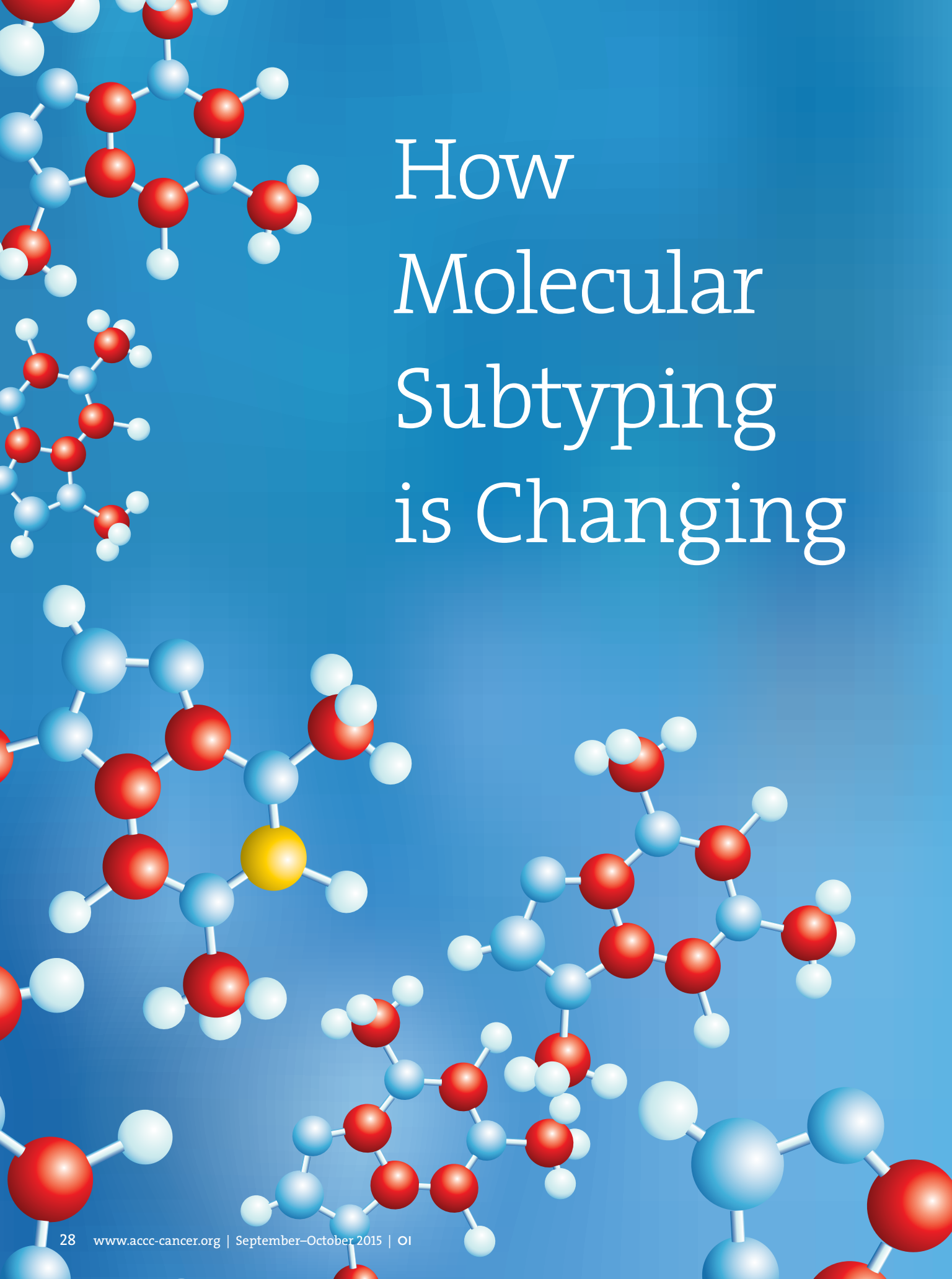
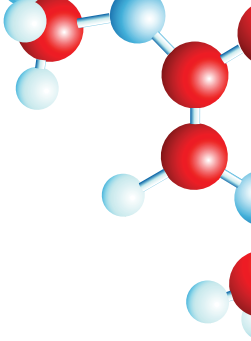


How Molecular Subtyping is Changing

BY JAMES V. PELLICANE, MD, FACS



Our Understanding of Breast Cancer

Our understanding of breast cancer continues to evolve with the release of every new study. One finding researchers have already confirmed: breast cancer is not just a *single* disease. Rather, breast cancer is a *category of diseases* made up of several different tumor types called molecular subtypes. Each subtype behaves differently, which in turn means each subtype may need to be treated differently to achieve the best outcome.

As the understanding of molecular subtypes evolves, it is becoming clear that the appearance of the cell based on traditional pathologic parameters, that is, IHC and FISH testing of estrogen receptor (ER), progesterone receptor (PR), and HER2 neu (HER2), may not always indicate the dominant pathway. While this finding is not news in itself, there are providers and cancer programs that have not yet integrated the results of the latest large studies of functional molecular subtyping. In other words, these providers and programs may be relying on diagnostic and treatment approaches that do not reflect the most recent findings. This is particularly true today of neoadjuvant treatment (i.e., preoperative), and will possibly encompass all breast cancer treatment in the coming years. Moreover, molecular subtyping is a component of precision medicine that is now becoming part of the national healthcare discussion.[1]

This article describes molecular subtyping and shows how it is changing both the understanding of breast cancer and how to treat it. The article summarizes the most important new studies and details the impact of this new information for community cancer centers.

Molecular subtyping is a component of precision medicine that is now becoming part of the national healthcare discussion.[1]

Molecular Subtyping 101

Molecular subtyping of breast tumors means grouping tumors according to their gene expression patterns. Subtyping can contribute to better outcomes, because different subtypes appear to have different prognoses and different responses to the various treatment alternatives, based on the functional pathway of the specific subtype.

Subtypes can be assessed using either clinical or molecular methods. Genomic tests for molecular subtyping include BluePrint

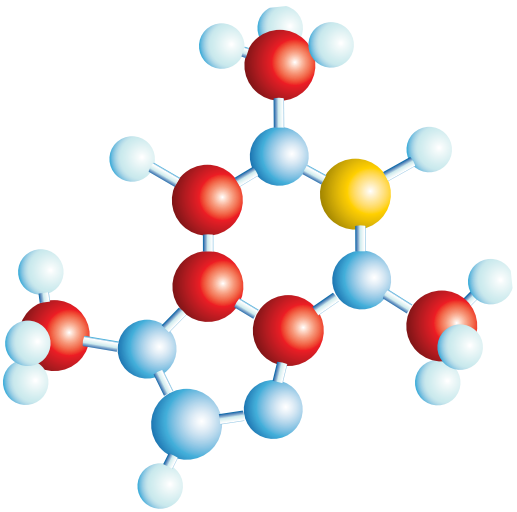
(Agendia, Inc.) and the PAM50 gene signature via Prosigna (Nanostring, Inc.). Prior to the availability of these molecular subtyping tests, different types of breast cancer have been distinguished by assessing the presence of the ER, PR, and HER2 biomarkers through standard assays, and by measuring the proliferation of the nuclear protein known as Ki-67, which is associated with cellular proliferation.

These standard assays examine the cell surface characteristics of the breast tumor to classify the tumor as a particular subtype. The two tests are known as IHC (immunohistochemistry) and FISH (fluorescence *in situ* hybridization). These tests are the current gold standard when assessing the presence of the above-mentioned biomarkers. But there has been some controversy over how to measure the biomarkers and how accurate those measurements are. Also, these measurements may not correlate with the dominant pathway that influences cell growth and cell survival.

IHC and FISH are considered complementary tests that pursue a similar goal: determining the presence or absence of the ER and PR receptors and if a tumor has extra copies of the HER2 gene. This latter gene makes proteins that act as receptors for certain signals that direct cell activity. In a healthy breast, the signals govern cell growth, division, and repair.

Extra copies of the HER2 gene are a red flag and may lead to uncontrolled cell growth. If a test result shows the tumor to have extra copies of HER2, the tumor is classified as "HER2-positive." If the test result is normal, the tumor is classified as "HER2-negative."

IHC and FISH tests are performed on a tumor sample from a core biopsy. Commonly, the IHC test will be used to determine ER and PR status. But sometimes the IHC test for HER2 assessment can be equivocal, which then prompts the pathologist to order the FISH test.



Inaccurate IHC-FISH results can have a profound effect on treatment recommendations and patient outcomes.

IHC-FISH testing is problematic for several reasons, including intrinsic problems with how the tests are conducted:

- The standards that establish criteria to determine whether a tumor is HER2-positive or negative continue to evolve
- If results are not clear-cut, individual pathologists may differ in their interpretations
- Sometimes, one part of a tumor can show up as HER2-positive, while another part tests as HER2-negative.

Inaccurate IHC-FISH results can have a profound effect on treatment recommendations and patient outcomes. For instance, if a tumor is incorrectly classified as HER2-negative, the patient may not be prescribed a drug, such as trastuzumab, which could help shrink the tumor before surgery.

Fortunately, the emergence of molecular subtyping means that a more accurate and reliable analysis of a tumor's subtype is now available. The 80-gene BluePrint subtyping assay, for example, is used in tandem with a test called MammaPrint, a 70-gene genomic assay that definitively stratifies patients as low-risk or high-risk for cancer recurrence. One of the advantages of this assay over other commercially-available tests is that it applies across all age groups, and is not restricted by estrogen or HER2 receptor status.

Some providers use a 21-gene test called Oncotype DX (Genomic Health, Inc.) to determine risk of recurrence. But the 21-gene test has a shortcoming: it does not always provide an absolute breakdown between low-risk and high-risk that the 70-gene test does. With the 21-gene test, more than one-third of patients receive an "intermediate" result that provides no clear indication about whether the cancer is likely to recur. Moreover, this test does not have any accompanying ability to provide molecular subtyping, nor is the test backed by the rigorous oversight reflected in an FDA clearance.

Both the 70-gene MammaPrint and the PAM50-based assays have achieved 510(k) clearance from the FDA. Both have also been acknowledged in the 2015 National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology as "clinically validated for prediction of [breast cancer] prognosis." (Note: Oncotype DX is also included in the 2015 NCCN Clinical Practice Guidelines in Oncology.)

Major Molecular Subtypes

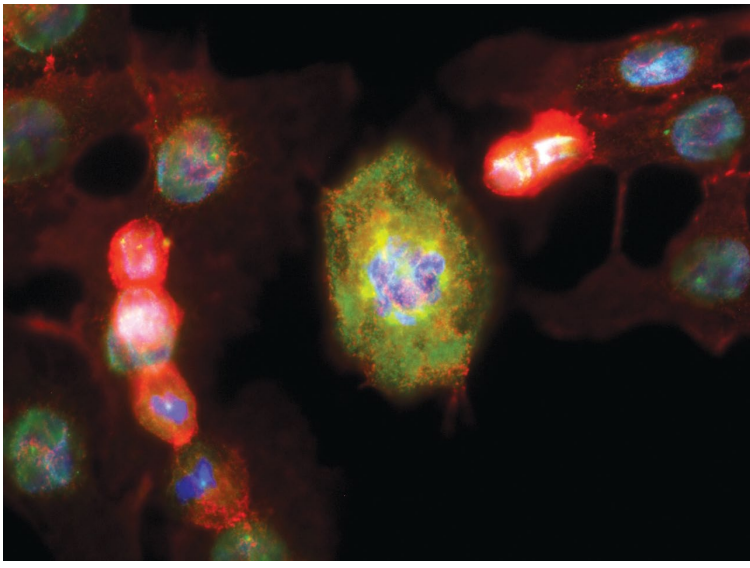
Again, there are two genomic tests for molecular subtyping: the BluePrint/MammaPrint combination and the PAM50 assay. Both BluePrint/MammaPrint and PAM50 identify four major subtypes:

Luminal A. Luminal breast cancers involve overexpression of the luminal epithelial cells that line the breast ducts and glands. When Luminal A breast cancers are identified by the BluePrint functional subtyping assay, it means these cancers are driven by the estrogen pathway and tend to be the least worrisome. These cancers grow slowly and, in most cases, can be successfully treated with limited surgery, radiation, and endocrine therapy—without chemotherapy. The cure rate is greater than 90 percent. Most breast tumors detected by screening mammograms are the Luminal A subtype.

Luminal B. Tumors of this subtype can be identified using one of the molecular subtyping technologies, for example, by a MammaPrint high-risk result in combination with a BluePrint Luminal result. While also driven by the estrogen pathway, these cancers are more concerning than Luminal A cancers because they tend to grow more aggressively. Chemotherapy is usually prescribed.

HER2. This subtype has extra copies of the HER2 receptor and, more importantly, is driven by the HER2 pathway. Although HER2-positive cancers are considered aggressive with the potential to recur, recent progress in treatment has increased the odds of a cure. In particular, targeted therapies, such as trastuzumab and pertuzamab, have been shown to be effective. The fact that targeted therapies can cure many HER2-positive cancers is an important reason to use genomic assays to more accurately identify the pathway driving them.

Basal. Basal tumors get their name because they involve overexpression of genes associated with basal-myoepithelial cells, which generally occupy a thin layer beneath the luminal cells. As these cancers are not driven by the estrogen or HER2 pathways, they typically do not have estrogen and progesterone receptors and do not feature an over expression of HER2. These cancers are aggressive, fast-growing tumors that have a substantial danger of spreading. Often these tumors are noted to be "triple negative." Although most triple negative breast cancers are of the basal subtype, not all basal subtype cancers are triple negative. In fact, about 20 percent of basal subtype patients are estrogen-receptor positive. By including them in the basal subtype group, they are added to a group that tends to respond better to chemotherapy. These tumors may not respond as well to endocrine therapy or drugs such as trastuzumab and pertuzamab, which are often prescribed for HER2-positive tumors.



Molecular Pathways in Human Breast Cancer Cells
Source: NCI Center for Cancer Research

The Latest Research

Recent studies support the accuracy and reliability of risk-recurrence and molecular subtyping assays.

NBRST. Among the important studies is the ongoing Neoadjuvant Breast Registry Symphony Trial (NBRST, pronounced "N-breast"), of which this author is a co-author.

Enrollment in this large, multi-site, prospective observational study is now closed, and some results have already been published.

For example, a study of 426 NBRST enrollees, published in the October 2014 issue of the *Annals of Surgical Oncology,* showed that 22 percent of patients who had been subtyped using IHC-FISH were reclassified and placed into more appropriate subtypes by the 70- and 80-gene assays. Commenting on the results, lead author and surgical oncologist Pat Whitworth, MD, noted that the study could especially affect the treatment of patients identified as "triple positive" in IHC-FISH. Roughly half of those patients do not exhibit HER2-type responses, the study found, so these patients might do better with a different treatment than would normally be given to an HER2-positive patient.

The study also concluded that neoadjuvant chemotherapy given to patients with Luminal A breast cancer (the most common subtype) will usually provide little if any benefit.[2] This finding confirms an earlier published study led by Stefan Gluck, MD.[3]

In a separate study of more than 300 patients, a similar percentage of patients (up to 25 percent) were more accurately classified by the 70- and 80-gene tests. This study was led by

medical oncologist Massimo Cristofanilli, MD, from Thomas Jefferson University. In this research, the genomic tests were compared to IHC alone.[4]

Finally, a prospective, outcome-based study confirmed the accuracy of the 70-gene test in stratifying breast cancer patients as either low- or high-risk for recurrence. In particular, the study showed that patients who received a low-risk score could safely choose to avoid chemotherapy and expect an excellent outcome, as measured at the five-year point.[5]

RASTER. This peer-reviewed study, called Microarray Prognostics in Breast Cancer (or RASTER), involved 427 breast cancer patients. Of the 219 patients who received a low-risk score, 85 percent decided not to receive chemotherapy. After five years, 95 percent of those patients were disease-free. The remaining 208 patients were determined by the 70-gene test to be at high-risk for recurrence. Of those patients, 81 percent received chemotherapy and 91 percent were disease-free after five years. The research, which was conducted in the Netherlands, was published in 2013 in *The International Journal of Cancer.*

Financial Implications of Molecular Subtyping

The primary benefits of molecular subtyping are obviously clinical, in terms of matching patients to the most appropriate treatment. Further, molecular subtyping may eventually enable patients to avoid side effects from treatments that will not really help them. But patients can also benefit financially from appropriate treatment-matching, as can the overall healthcare community.

Take, for example, patients whose IHC-FISH test results show they have an HER2 tumor. This is the type of breast cancer for which trastuzumab is usually prescribed. But molecular subtypes frequently identify these patients as Luminal subtype, suggesting that the ER pathway is driving the cell and that trastuzumab therapy may produce little or no benefit. If the NBRST findings are supported by further outcome studies, the savings from avoiding the cost of trastuzumab treatment could be substantial. Note: until further research is conducted, all of these patients should be treated with anti-HER2 therapy. In other words, it is a protocol that should remain in place until there is adequate outcome data based on molecular subtyping to change the existing protocol.

The potential financial benefits from improving how clinicians match patients to therapy can be extrapolated by looking at the number of women affected by breast cancer. In 2014 new cases of breast cancer totaled 232,670. Looking at this data another way, roughly 12.3 percent of women will be diagnosed with breast cancer during their lifetime, based on data analyzed from 2009 to 2011.[6]

Taking into account the cost of chemotherapy and other drugs used to treat breast cancer, one can estimate potential treatment costs. Today, the average brand-name drug used to treat cancer of any type is about $10,000/month (up from $3,000 in 2005).

> Molecular subtyping may eventually enable patients to avoid side effects from treatments that will not really help them.

Some cancer drugs cost three times that amount, or $30,000/month.[7] The cost of trastuzumab is not quite so steep, but a complete course, given over a year, can cost about $70,000 or nearly $6,000/month (as of 2012). Annual sales of the drug in 2011 were $5.5 billion.[8,9,10]

It is not uncommon for health insurers to require patients to pay 25 percent of their drug-related expenses.[11] Extrapolating from these data, a patient's out-of-pocket expenses responsibility could be estimated to be between $2,500 to $7,500/month for chemotherapy and about $1,500/month for trastuzumab.

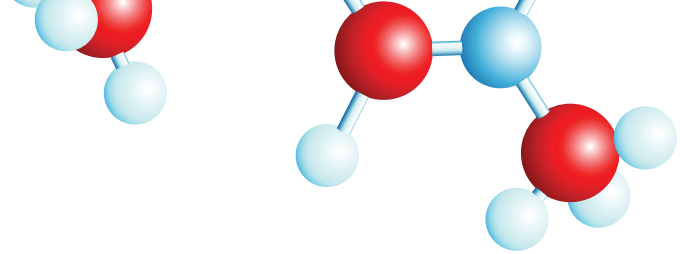
With more than 200,000 new breast cancer patients each year, you can see how the healthcare community as a whole could benefit financially if a large numbers of patients were able to avoid chemotherapy, while also avoiding potential side effects and the cost of treating those side effects.

A New Paradigm of Breast Cancer

Molecular subtyping offers a better way of individualizing breast cancer treatment. But the implications are bigger than that. By presenting a more nuanced view of breast cancer than clinical subtyping, molecular subtyping also suggests that our previous paradigm of breast cancer needs to be updated.

Before breast cancer subtyping (of any type) came along, patients would normally undergo surgery as their first treatment and then be referred to specialists for post-surgical radiation, or chemotherapy, or both.[12] Clinical subtyping via IHC/FISH made cancer specialists think differently about the whole treatment model, at least for some patients. First, clinicians understood that treatment needed to be matched to subtypes, since not all subtypes benefited from the same drugs. Second, clinicians understood that patients with certain subtypes benefited from treatment before surgery.

These findings also apply to molecular subtyping; however, the research community does not yet have longer-term outcomes from molecular subtyping studies. The benefit has so far been seen neoadjuvantly in patients who have a pathological complete response (pCR), meaning they have no measurable cancer after treatment that is informed by molecular subtyping. Pre-surgical treatment for HER2-positive patients with trastuzumab can sometimes destroy all traces of the disease so that only limited



surgery is needed to prevent recurrence.[13] Pre-surgical elimination of detectable cancer also makes breast reconstruction after surgery easier.[13] Today, clinicians frequently view this pCR outcome as a surrogate for a favorable long-term outcome.

Now that studies such as the NBRST Trial are consistently showing molecular subtyping to be more accurate than IHC-FISH, it is probably time to again revise our understanding of breast cancer. A paradigm based on IHC-FISH defines subtypes of breast cancer based on whether certain receptors are overly represented on a tumor cell's surface, indicating overexpression of an associated gene. Molecular subtyping demonstrates that this way of looking at cancer may be inadequate. To understand how a tumor is actually behaving, one has to examine the molecular profile and identify the genes driving that behavior.

Understanding which genes are driving a tumor's behavior, in turn, may eventually change the treatment paradigm. It could potentially provide more accurate information than IHC-FISH about which treatments will be effective in the long-term and which treatments will not. With pre-surgical treatment having assumed a greater role in cancer treatment, this difference is even more important than it would have been in the days when surgery always preceded drug therapies.

This evidence suggests the eventual arrival of a new treatment paradigm in which tumors are classified by molecular subtype/chemosensitivity so that patients and their physicians can make better-informed decisions about whether pre-surgical chemotherapy will be helpful. According to NBRST data, about 20 percent of HER-positive breast cancers are reclassified as basal subtype, placing them into a more chemosensitive group compared to a clinical luminal subtype. This data has clinical utility today—because it does not involve withholding therapy, but instead identifies more aggressive subtypes that will benefit from more aggressive treatments.

Patient & Programmatic Benefits

Incorporating molecular subtyping into daily practice creates multiple advantages for breast cancer patients, cancer programs, and the overall healthcare community, including:

- In the future, certain patients may benefit from receiving neoadjuvant treatment that is based on greater knowledge of their cancer and is more targeted for their tumor's molecular subtype. Patients may also be able to avoid treatments that are shown to be less effective for their subtype as we continue to accumulate more data, specifically outcomes data relating to specific therapies.
- Cancer programs can both update and expand the services they offer patients. Analyses of tumors' molecular subtypes are as readily available to community cancer centers as they are in the academic setting.
- The healthcare community benefits if molecular subtyping generates refined treatments that are more effective in combating breast cancer. In the future, molecular subtyping may help reduce the number of expensive treatments that are shown to be ineffective for certain tumor subtypes, potentially resulting in substantial cost savings.
- Genomic tests are accessible by any cancer program, no matter its size (large or small) or location (rural or urban). Just as important, payers are now educating themselves about these tests and starting to support the technology.
- Because molecular tests are performed on breast biopsy tissue, these tests do require an extra procedure. Insurance coverage for genomic tests is a developing situation, but it is headed in the right direction, with widespread coverage for both the 21-gene and 70-gene assays.

It is hard to make predictions about what lies farther down the road with regards to breast cancer research and treatment, but certain trends seem clear. Because genomic testing for breast cancer is a relatively new and immensely promising field, it is rich in ongoing research. Future research will help providers to better individualize the treatments prescribed for breast cancer patients. As these improvements are made, outcomes will improve, too, and the medical and insurance communities will more fully embrace the progress. But that does not mean cancer programs should wait to use this technology. Molecular subtyping is sufficiently advanced to be helping patients right now. For example, clinicians should consider using molecular subtyping for any newly diagnosed patient with Stage I or Stage II invasive breast carcinoma that is lymph-node-negative or lymph-node-positive.

What Might the Future Hold?

New research will lead to further division of the four-subtype scheme used today. For instance, there are indications that the HER2-positive subtype may actually consist of two or three separate types of breast cancer, each with a different response to chemotherapy. The same may be true of Luminal B cancers.

Treatment for the HER-positive, basal subtype is an evolving situation.

Some HER2-positive and Luminal cancers do not respond to pre-surgical chemotherapy. What clinicians do not know for certain is if that's because those patients would have a poor outcome anyway or because anti-HER2 therapy is unnecessary.

Current research should yield stronger outcome data about molecular subtyping. Once we have that data, clinicians may be able to take full advantage of the latest research findings, including some of the data coming out of the NBRST study.

It is a new day for breast cancer analysis and treatment. Genomic tools are making a difference in many breast cancer patients' lives. While there is so much more to learn and apply,

TWO PATIENT CASE STUDIES

Here are some examples of how molecular subtyping has affected actual patients.

At age 39, Kara S. of Nashville discovered a lump in one of her breasts. IHC-FISH testing showed her tumor to be "triple-positive," positive for overproduction of HER2, ER, and PR receptors. But molecular subtyping revealed that the IHC-FISH subtyping was wrong. She received neoadjuvant treatment based in part on the genomic analysis, and the treatment was successful, meaning there was no invasive carcinoma detectable in her breast or axilla (underarm) before surgery.

Susan B. was 52 when her cancer was discovered. After her tumor was analyzed with IHC-FISH, test results gave no clear indication of the tumor subtype. Molecular subtyping showed that Susan had a basal tumor with a high risk of recurrence, which helped Susan and her physician to make a well-informed treatment decision. She was given pre-operative chemotherapy and, similar to Kara S., had a complete pathologic response (no apparent remaining cancer) to the treatment.

This kind of pathologic complete response to pre-operative therapy is believed to predict a highly favorable outcome for the patient.

that is no different from the state of knowledge with any form of cancer. Thanks to molecular diagnostics, outcomes and many patients' disease-related life experiences are already much improved. Because of all the ongoing research into genomic testing, those outcomes and experiences will only get better in the years to come. OI

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